



# Queen Control or Queen Signal in Ants: What Remains of the Controversy 25 Years After Keller and Nonacs' Seminal Paper?

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## Abstract

Ant queen pheromones (QPs) have long been known to affect colony functioning. In many species, QPs affect important reproductive functions such as diploid larvae sexualization and egg-laying by workers, unmated queens (gynes), or other queens. Until the 1990s, these effects were generally viewed to be the result of queen manipulation through the use of coercive or dishonest signals. However, in their seminal 1993 paper, Keller and Nonacs challenged this idea, suggesting that QPs had evolved as honest signals that informed workers and other colony members of the queen's presence and reproductive state. This paper has greatly influenced the study of ant QPs and inspired numerous attempts to identify fertility-related compounds and test their physiological and behavioral effects. In the present article, we review the literature on ant QPs in various contexts and pay special attention to the role of cuticular hydrocarbons (CHCs). Although the controversy generated by Keller and Nonacs' (Anim Behav 45:787–794, 1993) paper is currently less intensively debated, there is still no clear evidence which allows the rejection of the queen control hypothesis in favor of the queen signal hypothesis. We argue that important questions remain regarding the mode of action of QPs, and their targets which may help understanding their evolution.

**Keywords** Cuticular hydrocarbons · Fertility signal · Signal honesty · Signal perception · Social insects · Sociobiology

## Introduction

The Formicidae are characterized by great diversity in social organization. Colonies can contain one or multiple reproductive females and tens to millions of workers who

care for the brood, forage, and defend the colony. In most species, queens attract workers, who form a retinue around them. In addition, a queen's presence has multiple consequences for a colony's reproductive output: it inhibits egg-laying by workers and gynes (winged virgin queens), reduces the fertility of nestmate queens, and regulates diploid brood sexualization. Myrmecologists have long recognized the role of queen pheromones (QPs) in mediating these effects. However, until the 1990s, ant colonies were generally viewed as top-down regulated systems in which queens had full control over the workers and the brood. It was believed that queens used pheromones to manipulate nestmates' behavior and physiology against their own interest (Röseler 1991).

In 1993, Keller and Nonacs published "*The role of queen pheromones in social insects: queen control or queen signal?*". In this paper, they questioned the existing hypotheses regarding the evolution of QP-mediated effects in social insects. They argued that, although it was possible that queens manipulate workers and brood through

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This review is dedicated to Prof. Luc Passera for his enormous contribution to the knowledge of ant biology that inspired generations of myrmecologists and sociobiologists.

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behavioral interactions, chemical control is much less likely. More than a decade earlier Seeley (1979, 1985) had already challenged the idea that honeybee queens used their pheromones to manipulate the hive. Keller and Nonacs (1993) expanded this concept to other social insects and proposed a general framework to study the evolution of QPs. They reasoned that queen chemical control could have evolved either because QPs were coercive or because they dishonestly signaled a queen's quality. In both cases, the result would be a modification of the behavior and/or physiology of a queen's nestmates against their own interest. However, in the first case, the production of a coercive pheromone would inevitably result in selection for resistance, so that, to retain control, queens would have to continuously increase their pheromone production or diversify its chemical composition. Eventually, the cost for queens of this chemical arms race would probably outweigh the benefit of their reproductive monopoly. Moreover, queens would have to be immune to their own QPs. In the second case, the evolution of a dishonest signal could be counteracted fairly easily if workers had alternative reliable sources of information, such as egg number, to evaluate queen quality or presence.

Keller and Nonacs proposed an alternative hypothesis inspired by signaling theory and deeply rooted in evolutionary biology. They argued that QPs are honest signals directed to nestmates which enable them to behave in a way that maximizes their own inclusive fitness. At that time, a QP had only been identified in the honeybee (*Apis mellifera*). This paper provoked intense controversy and stimulated many attempts to identify QPs in other social insects and to determine the precise mechanisms by which they affect colony reproductive functions. In particular, the diversity in the social organization of ants provided an excellent system with which to initiate comparative analyses and test predictions related to the honesty of QPs in various contexts. This research was further facilitated by the development of novel and powerful technologies, like capillary gas chromatography and the analysis of microsatellite markers, which allowed joint studies of ant chemistry and social systems.

In this controversy, proximate effects of QPs on receivers' physiology and behavior have not always been well differentiated from ultimate control of receivers' fitness by queens (Peso et al. 2015). Although recent modeling suggests that queen control may theoretically evolve under certain conditions (Olejarz et al. 2017), there seems to be a large consensus in current literature that QPs are honest, rather than coercive or dishonest, signals (Grüter and Keller 2016; Heinze and d'Etorre 2009; Smith and Liebig 2017). However, most conclusions are based on inferences and, to our knowledge, no experimental evidence clearly rejects the hypothesis of queen control in

ants or other social insects. As argued by Keller (2009) and Grüter and Keller (2016), important gaps in our knowledge of the chemical nature and physiological functioning of QPs hamper a good understanding of their evolution. Here, we review the literature on QP-mediated effects in ants. We first present the major contexts in which QPs affect colony reproduction. Second, we review studies on the sources of QPs and evaluate current evidence regarding the specific role of cuticular hydrocarbons (CHCs). Finally, we analyze current knowledge on the physiological and molecular effects of QPs in ants and finish by identifying questions that should be addressed in the future. Throughout this article we will refer to QPs using the definition of Holman et al. (2010a): a chemical or chemical blend that "i) characterizes the reproductive(s) and (ii) induces behavioral and/or physiological responses in other colony members".

## Pheromone-Mediated Effects on Reproduction and Development

### Regulation of Worker Egg-Laying

It has been known for many years that ant QPs influence the regulation of worker egg-laying (Bier 1954; Hölldobler and Wilson 1983; Passera 1969, 1980a). In many species, workers have functional ovaries but generally refrain from laying viable eggs when they perceive the presence of a queen—in some species, queenright workers lay trophic eggs that are consumed by the queen or the brood (Dietemann and Peeters 2000; Hölldobler and Carlin 1989;). In contrast, when no queen is present, workers readily lay arrhenotokous haploid eggs that yield males or, more rarely, thelytokous diploid eggs that yield workers or queens. Two hypotheses explain why workers collectively refrain from laying eggs when a queen is present in the colony. First, in polyandrous colonies, workers are genetically more related to the queen's sons than to other workers' sons and should therefore favor the former over the latter (Ratnieks 1988; Wenseleers and Ratnieks 2006; Woyciechowski and Łomnicki 1987). However, worker policing is not restricted to polyandrous species and occurs even if the genetic relatedness of workers to their sisters' sons is high [see studies of *Platythyrea punctata* (Hartmann et al. 2003) and *Aphaenogaster japonica* (Iwanishi et al. 2003)]. A second, more general explanation may be that worker egg-laying reduces colony-level productivity, which negatively affects all individuals (Hammond and Keller 2004; Heinze 2004; Ratnieks 1988). Colony level costs of worker reproduction have rarely been accurately measured and could potentially include a reduction in the efficiency of other tasks performance (like foraging, colony defense etc.) or an increase of overt aggression among workers. To our knowledge, the best

estimation of the cost of worker reproduction was conducted in *Diacamma* sp. in which it provoked a reduction of worker life span by up to 88% (Tsuji et al. 2011).

Efficient mutual control prevents selfish behaviors and selects for self-restraint in queenright conditions. Hence, in addition to their negative effects on worker ovarian development and egg-laying, QPs trigger the punishment of egg-layers and/or egg elimination. There are now numerous reported examples of severe aggression specifically directed by queenright workers toward their egg-laying nestmates [see studies of *Novomessor (Aphaenogaster) cockerelli* (Hölldobler and Carlin 1989), *Rhytidoponera confusa* (Crosland 1990), *Gnamptogenys menadensis* (Gobin et al. 1999), *Harpegnathos saltator* (Liebig et al. 1999), *Myrmecia gulosa* (Dietemann et al. 2005), *Temnothorax* sp. (Brunner et al. 2011; Stroeymeyt et al. 2007), *Pachycondyla inversa* (van Zweden et al. 2007), and *Aphaenogaster senilis* (Ichinose and Lenoir 2009; Villalta et al. 2015)]. In contrast, in some species, like *Formica fusca*, punishment of egg-layers is rare and workers lay numerous haploid eggs even in queenright conditions. However, colonies of this species predominantly produce diploid adults and very few males, which suggests that most worker-laid eggs are removed by other workers (Helanterä and Sundström 2005). It is not known in *F. fusca* if eggs are removed because of their parentage or their gender, but recognition of egg parentage has been shown in a few other species [e.g., *Dinoponera quadriceps* (Monnin and Peeters 1997), *Diacamma* sp. (Kikuta and Tsuji 1999), *P. inversa* (D'Ettorre et al. 2004), and *Camponotus floridanus* (Endler et al. 2004, 2006)].

### Regulation of Wing-Shedding and Egg-Laying in Gynes

In many ant species, gynes remain in their mother colony until they mate and become reproductively active. Mating is followed by wing-shedding, which further stimulates ovarian development and egg-laying (Jemielity et al. 2006). In the red imported fire ant, *Solenopsis invicta*, the presence of a fertile queen in a colony inhibits wing-shedding and haploid egg-laying in gynes (Fletcher and Blum 1981). Conversely, when the queen is removed, gynes rapidly shed their wings and initiate haploid egg-laying. Fletcher and Blum (1981) showed that the regulation of wing-shedding and ovarian development is mediated by a “relatively nonvolatile primer pheromone (or pheromones) produced by the mated queen and distributed by the workers”. Interestingly, virgin dealated gynes readily become attractive to workers (Glancey et al. 1981) and also inhibit wing-shedding in other gynes (Willer and Fletcher 1986), which suggests that dealation triggers the production of QPs. Subsequent results have shown that pheromone production in *S. invicta* correlates positively with the physical and

physiological state of gynes (Obin et al. 1988), suggesting the pheromone is an honest signal. Moreover, although egg-laying gynes are well tolerated by queenless workers, they are violently harassed by queenright workers, probably for the same reason that queenright workers punish egg-laying workers in other species (Keller and Nonacs 1993; Vargo 1999). The benefit for queenless workers in allowing egg-laying by gynes may be enhanced by the fact that fire ant workers have lost their ovaries and cannot lay their own male eggs. Similarly, gynes of the Argentine ant, *Linepithema humile*, shed their wings and start laying eggs when they are queenless (Passera and Aron 1993). Yamauchi et al. (2007) also showed that queens of *Cardiocondyla* sp. inhibit wing-shedding in gynes. These authors argued that this is due to violent aggression directed by queens towards gynes, although the methods that were used in this study do not preclude a role for QPs in this process.

### Queen-Queen Competition

Queen-queen competition occurs in both permanently and temporarily polygynous colonies. Permanent polygyny results from the adoption of newly mated queens into adult colonies, whereas temporary polygyny (pleometrosis) occurs when several, generally unrelated, queens form a colony together. Queen-queen competition in permanently polygynous colonies can manifest itself as a decrease in queens' fecundity with an increase in their density [e.g., *Plagiolepis pygmaea* (Mercier et al. 1985), *L. humile* (Keller 1988b), and *S. invicta* (Vander Meer et al. 1992; Vargo 1992)]. In pleometrosis, queens tolerate each other well and can cooperate to increase the success of colony founding. However, once the first workers have emerged, intensive fights occur among queens and between queens and workers until only one queen remains. Queen-queen competition may be provoked by i) exploitative competition, whereby the food share received by each queen arithmetically decreases as their number increases, or ii) interferences mediated by direct and indirect behavioral and/or chemical interactions.

In *S. invicta*, QPs may play a role in queens' ability to directly inhibit each other's reproduction. In this species, the addition of freshly killed queen corpses to a colony reduces the fertility of living queens (Vargo 1992). However, it is not clear if queen corpses directly affect other queens' fecundity or if the corpses simply occupy workers' interest, with the effect of reducing the amount of care received by living queens. An attempt to link QPs with a decrease in per capita egg-laying of co-habiting queens of *Leptothorax acervorum* was inconclusive (Bourke 1993). Likewise, in *L. humile*, highly fecund queens occur with equal frequency in both monogynous and polygynous conditions, which suggests a lack of chemical competition among queens (Keller 1988a).

However, no rigorous behavioral and/or chemical tests of pheromone-mediated queen-queen competition has been undertaken in that species.

It is likely that pheromone-mediated queen-queen competition occurs through direct effects on workers. In *L. humile*, queen attractiveness to workers is lower in polygynous than in monogynous colonies. Moreover, queen attractiveness correlates with fertility, so that polygynous queens have lower fecundity and weigh less than monogynous queens, probably because the former receive less food from workers (Keller 1988a). In *S. invicta* the most attractive queens receive more food from workers via trophallaxis and, in consequence, have a higher ovipositional rate (Chen and Vinson 2000). In polygynous colonies of *Leptothorax* sp. A and *F. fusca* workers direct more care to the more fecund queens (Ortius and Heinze 1999 and Hannonen et al. 2002, respectively). Finally, in pleometrotic colonies, workers are unable to estimate their degree of relatedness with queens and cannot favor their own mother. Instead, they may rely on QPs to identify the most productive queen, who is also statistically the most likely to be their mother. This was supported by a study on the European black garden ant *Lasius niger*, in which queens' ovarian development was shown to correlate with pheromone production and queen survival rate during the queen elimination phase (Holman et al. 2010a). Furthermore, it has been proposed that QPs may serve as a regulatory mechanism to adjust queen reproductive output with respect to the presence of other queens (Holman et al. 2013b).

In *S. invicta* and *L. humile*, colony queen number is regulated by the adoption and execution of queens, two processes that also seem to be mediated by QPs. Fletcher and Blum (1983) proposed that when QP concentration in *S. invicta* colonies exceeds an optimal range, workers execute the lower-ranking queens of a pheromone hierarchy. Likewise, QPs of this species prevent the adoption of newly mated queens by increasing worker aggression against the new queens (Vander Meer and Alonso 2002). A similar pattern has been reported in *L. humile*, in which queenless colonies are more likely to accept non-nestmate queens than queenright colonies are (Vasquez et al. 2008).

### Regulation of Larval Caste Fate

The mechanisms underlying caste determination in ants are still poorly understood. In a few species, caste fate is determined at the egg stage through genetic or epigenetic effects (Helms Cahan et al. 2002; Leniaud et al. 2012; Passera 1980b), which gives queens great power over the timing and amount of gyne production. However, in most species, young diploid larvae are totipotent until they enter into either queen- or worker-developing routes which depends on worker-controlled environmental factors. Regardless of the mechanisms involved, it is not in the interest of the workers or the

queen to allocate excessive resources to gyne production, because the production of many gynes in the presence of a mother queen tends to debilitate the colony and jeopardizes future reproduction (Bourke and Ratnieks 1999). The negative effect of excess gyne production is exacerbated in species with dependent colony founding—i.e. when the newly mated queens do not found new colonies on their own but need the help of workers. This is because, in these species, the newly mated queens disperse over very short distances which enhances local resource competition. However, when there is no queen, workers may improve their fitness by diverting colony resources to favor the development of the youngest diploid larvae into gynes rather than into sterile workers. This may be particularly true if one of the produced gynes replaces her absent mother. Extreme QP-mediated inhibition of gyne production occurs in army and gypsy ants, in which gynes are only produced in queenless or very large queenright nests (Schneirla 1971; Ledoux and Dargagnon 1973; Boulay et al. 2007). Similarly, in the polydomous colonies of the desert ant *Cataglyphis iberica*, gynes are produced in satellite nests that are far from the queen's influence (Cerdá et al. 2002), and in *L. humile*, gynes only develop after the spring elimination of more than 90% of the queens by workers (Keller et al. 1989; Vargo and Passera 1991).

Numerous examples have shown that regulation of caste development depends on QPs [(e.g., *Myrmica rubra* and *M. scabrinodis* (Brian and Carr 1960; Brian 1970), *Temnothorax* (*Leptothorax*) *nylander* (Plateaux 1971), *A. senilis* (Boulay et al. 2009; Ledoux and Dargagnon 1973; Ledoux 1976; Ruel et al. 2013a), *M. pharaonis* (Berndt 1975; Boonen and Billen 2017; Edwards 1987), *Plagiolepis pygmaea* (Passera 1980a), *Cataglyphis cursor* (Cagniant 1988), *S. invicta* (Vargo and Fletcher 1986), and *Linepithema humile* (Passera et al. 1995; Vargo and Passera 1991)]. However, the causal link between QPs and caste development is not as clearly understood in ants as it is in the honeybee, for example. One hypothesis is that QPs affect workers' behavior: workers reduce the amount of food they provide to each larva, thus ensuring that larvae develop into workers (Amor et al. 2016; Smith et al. 2008b; Smith and Suarez 2010). However, the modification of worker feeding behavior may be either the cause or a consequence of larval development into gynes. For example, larvae of *Cataglyphis tartessica* can develop into worker-size gynes regardless of how they were fed by workers, which suggests that the production of queen-specific organs does not depend exclusively on food quantity (Amor et al. 2011, 2016).

Another hypothesis is that QPs affect the quality of worker-processed food. In many species, workers feed larvae through trophallaxis and/or trophic eggs. A pheromone-induced modification of these secretions could thus direct larval development. This hypothesis is supported by the recent finding that during trophallaxis, workers of *C. floridanus* transfer a fluid that contains regulating

factors and juvenile hormone (JH), which affect larval growth and potentially caste fate (Leboeuf et al. 2016).

Finally, queen pheromones may stimulate social control of larval caste fate by promoting soft biting of gyne-developing larvae (Penick and Liebig 2012, 2017) or more directly through their cannibalism (Boonen and Billen 2017; Vargo and Fletcher 1986; Edwards 1991b; Vargo and Passera 1991; Villalta et al. 2016).

## The Sources of Ant QPs

### Abdominal Glands

Ants possess numerous exocrine glands with which they synthesize hundreds of compounds. However, chemical ecologists have long been unsuccessful in identifying the source and chemical composition of ant QPs (Passera 1980a). The first reported source of a worker-attracting QP was the poison gland of *S. invicta* (Vander Meer et al. 1980). The contents of this gland also inhibit gyne wing-shedding and ovarian development (Vargo 1997) and stimulate cannibalism of sexual larvae (Klobuchar and Deslippe 2002). The chemical mixture contains hydrocarbons and large amounts of caste-specific piperidine alkaloids that are less abundant in gynes and workers than in queens (Brand et al. 1973; Eliyahu et al. 2011). However, the respective roles of these compounds as QPs are doubtful and deserve reevaluation (Vander Meer et al. 1980). Furthermore, Vargo and Hulsey (2000) showed that gynes whose poison sac had been experimentally removed still inhibited dealation in other gynes, which suggests that there are multiple exocrine sources of the QP. In contrast to *S. invicta*, the poison sacs of gynes of *A. senilis* contain more alkaloids than those of same-age queens, which may suggest that, in this species, alkaloids may be involved in sexual behavior rather than in the regulation of reproduction (Ruel et al. 2013a).

Ant Dufour glands generally contain volatile compounds such as short-chain hydrocarbons, fatty acids, alcohol, esters, and ketones, among others. The role of these chemicals as QPs has been studied in only a few species and the results are inconsistent. For example, the contents of queen Dufour glands are highly attractive to workers in *Monomorium pharaonis* (Edwards and Chambers 1984), but not in *Myrmecia gulosa* (Dietemann et al. 2003). However, an interesting function of the Dufour gland secretions was discovered in some ants in which they are used by queens to signal their opponents and maintain their supremacy. For example, in *Leptothorax gredleri*, dominant queens apply Dufour gland secretions on their opponents which triggers aggression by workers (Heinze et al. 1998). Another example was well documented in the ponerine species *Dinoponera quadriceps*.

Over the course of evolution, this species has lost the queen caste: all workers have a spermatheca but only one behaviorally dominant individual, the gamergate, mates and reproduces. The gamergate's reproductive monopoly is often challenged by high-ranking subordinate workers, who are punished by low-ranking workers. To maintain her supremacy, the gamergate uses her Dufour gland to tag challengers, which stimulates aggression by low-ranking workers (Monnin et al. 2002). A similar phenomenon was found in *N. cockerelli*, in which the queen sprays the content of her Dufour gland on workers that have developed ovaries (Smith et al. 2012a). Likewise, queens of *A. senilis* are severely aggressive towards gynes and while fighting, will deposit large amounts of Dufour gland secretions on them, which provokes aggression by workers (Boulay, unpublished data).

### Cuticular Hydrocarbons

Long-chain saturated and unsaturated hydrocarbons form the cuticle of most terrestrial arthropods and have evolved as protection against desiccation. However, in some species, they have been coopted for intra- and interspecific communication (Blomquist and Bagnères 2010). For example, in *D. quadriceps*, the relative proportions of some CHCs correlate with ant fertility. By using a non-destructive method of extraction, Peeters et al. (1999) showed that gamergates have a much larger amount of 9-hentriacontene than subordinate non-fertile workers do (Peeters et al. 1999). Further behavioral studies suggested that low-ranking workers can use 9-C31:1 as a reliable fertility signal to punish challengers who develop ovaries and exhibit intermediate levels of this hydrocarbon. Other correlations between ovarian development and the production of specific CHCs have since been shown in multiple species: *Harpegnathos saltator* (Liebig et al. 2000), *Diacamma ceylonense* (Cuvillier-Hot et al. 2001, 2002), *F. fusca* (Hannonen et al. 2002), *M. gulosa* (Dietemann et al. 2003), *L. humile* (Abril et al. 2018 in press; De Biseau et al. 2004), *Streblognathus peetersi* (Cuvillier-Hot et al. 2004), *Gnamptogenys striatula* (Lommelen et al. 2006), *S. invicta* (Eliyahu et al. 2011), *Hypoponera opacior* (Foitzik et al. 2011) and *Pachycondyla verenea* (Evison et al. 2012). Interestingly, in *L. humile*, a recent study showed that patterns of queen CHCs correlate with ovarian development and enable predictions of individual queens' survival during the queen-elimination phase that is typical of this invasive species (Abril et al. 2018).

In other species, CHC profiles correlate with mating status. In *Ectatomma tuberculatum*, queens exhibit a 250-fold increase in heptacosane quantity compared to gynes, which provokes a visible modification of the cuticle (Hora et al. 2008). Similarly, 30 min after mating, *Leptothorax gredleri* queens already show a significant increase in the proportion of linear alkanes and a decrease in dimethylalkanes (Oppelt and Heinze

2009). The opposite result was found in *A. senilis*, in which queens have a greater relative quantity of long-chain dimethylalkanes on their cuticle than same-age gynes do (Ruel et al. 2013a). In a recent study, Monnin et al. (2018) found significant differences in the composition of CHCs and Dufour gland contents among queens, gynes, and workers of *C. cursor*. Although these differences may serve to signal the fertility of the newly-mated queens to workers, they may also act to repel males and prevent further mating attempts.

Experimental tests demonstrating that workers recognize and respond to queen-specific CHCs have only been provided in a handful of species. In *N. cockerelli* and *Odontomachus brunneus*, queens and queenless egg-laying workers typically produce large amounts of pentacosane and 9-nonacosene, respectively (Smith et al. 2008a, 2012b). When coated on non-egg-laying queenright workers, these hydrocarbons provoked aggression from other workers that was similar to that received by egg-laying workers, which reveals their involvement in the process of punishment (Smith et al. 2009, 2012b). In *Lasius niger*, *L. flavus*, and *C. iberica*, several linear and 3-methylalkanes are overrepresented on queens' cuticles. When synthetic hydrocarbons were experimentally provided to queenless colony fragments for several weeks, worker egg-laying was delayed, which clearly indicated that these compounds are involved in the queen-mediated regulation of worker egg-laying (Holman et al. 2010a, b, 2016a; Van Oystaeyen et al. 2014). In *L. niger*, different enantiomers of 3Me-C31 have effects on worker physiology and behavior: when queenless colonies were treated with synthetic *R*- and *S*-enantiomers separately or in a racemic mixture, workers refrained from developing ovaries, as occurs under queenright conditions. However, only the colonies treated with the *S*-enantiomer showed a reduction in aggressive interactions (Motais de Narbonne et al. 2016), which suggests that aggression and the development of ovaries are likely independent effects in this species.

### Egg-Marking Pheromones

The destruction of worker- and gyne-laid eggs in some species suggests that queen-laid eggs are marked with QPs but worker- and gyne-laid eggs are not. For example, *S. invicta* queens deposit alkaloids from their poison glands on eggs during oviposition (Vander Meer and Morel 1995). These compounds have an antimicrobial function. Moreover, the QPs seem to rapidly attract workers to take care of the eggs. These compounds could also enable workers to assess egg parentage, potentially ensuring the destruction of gyne-laid eggs. In *D. quadriceps*, the gamergate-specific hydrocarbon 9-C31:1 is also present on gamergate-laid eggs, and the amount of 9-C31:1 on the eggs correlates with the amount on the egg-layers' cuticles (Monnin and Peeters 1997). Similarly, CHCs found predominantly in queens form the

tegument of eggs of *Pachychondyla inversa* (D'Ettorre et al. 2004; van Zweden et al. 2009), *Camponotus floridanus* (Endler et al. 2006), and *A. senilis* (Ruel et al. 2013b). Such egg-marking likely helps workers identify and destroy eggs that were not laid by the queen.

Another function of egg-marking has been shown in *C. floridanus*, which forms very large nests. Queen-laid eggs serve as a message to workers all over the nest that a queen is present in the colony, which deters them from developing ovaries (Endler et al. 2004). Hölldobler and Carlin (1989) suggested that this also occurs in *N. cockerelli*. In *M. pharaonis*, the addition of queen-laid eggs to queenless colonies prevents larvae from developing into gynes (Edwards 1987). However, if the queenless colonies have already started to produce gynes, the addition of queen-laid eggs does not affect their survival (Boonen and Billen 2017). In queenless colonies of other species, queen-laid eggs do not prevent gyne production, which may indicate that the effects of egg-marking are species or context-specific (Ruel et al. 2013b; Vargo and Fletcher 1986).

### The Evolution of QPs from Ancestral Functions in Solitary Insects

There are two hypotheses for the evolution of CHCs as honest signals. First, according to Zahavi's handicap theory (Zahavi 1975), their production should be costly for the queens (Heinze and d'Ettorre 2009). Presumably the over-production of some CHCs has a cost for the queens. However, we still know too little about hydrocarbon metabolism in insects to accurately estimate this cost, particularly as the metabolic cost might reflect not only the quantity but also the quality of the hydrocarbons involved (van Zweden et al. 2009). Another problem is that over-production of some CHCs may affect other cuticle functions like resistance to desiccation and protection against pathogens. Despite the costs involved, the maintenance of diverse biosynthetic routes to produce various compounds is likely to enhance the evolutionary stability of the trait.

Second, if queen CHCs are honest signals, one would expect them to be under strong stabilizing selection. In contrast, the manipulative queen control hypothesis predicts a great diversification of QPs as a consequence of the worker-queen evolutionary arms race (Brunner et al. 2011; Heinze and d'Ettorre 2009; Keller and Nonacs 1993). In a recent study, van Oystaeyen et al. (2014) suggested that the role of CHCs as QPs was highly conserved in eusocial insects and probably evolved from sexual pheromones in solitary ancestors; within ants, however, queen-specific compounds show a great structural diversity (van Zweden et al. 2009). In some species, queen-specific CHCs are linear-, monomethyl-, or dimethylalkanes, with either relatively short or relatively long chains, while other species use unsaturated compounds. An

interesting approach to study the evolution of QPs was employed by Brunner et al. (2011). These authors tested the effects of *Temnothorax* queens on the ovarian development of workers of various closely related species. They found that the queen effects did not strictly match the phylogeny, which does not support strong conservatism of QPs. In contrast, (Holman et al. 2013a) showed that 3-MeC31 from *Lasius* inhibited ovarian development across several species. We believe that generalizing this approach would provide greater understanding of QP evolution.

## Molecular Effects of QPs

### Perception of QPs

Electroantennography conducted in *P. inversa* has revealed that workers respond specifically to the putative QP of this species (D'Ettorre 2004). QPs and other chemicals are detected by sensory hairs (sensilla) located on chemosensory organs, especially the antennae. In carpenter and leaf-cutting ants, female-specific olfactory sensilla basiconica are highly innervated by up to 130 olfactory receptor neurons (ORNs) (Kelber et al. 2010; Nakanishi et al. 2010) that are implicated in CHC chemoperception (Kidokoro-Kobayashi et al. 2012; Ozaki et al. 2005). Several lines of evidence suggest that QP perception is highly specific and plastic over both time and reproductive stages. In *C. floridanus*, Sharma et al. (2015) showed that certain sensilla basiconica are sensitive to queen CHCs and that, using these structures, workers were able to detect and distinguish enantiomers of a proposed QP (3-methylheptacosane). In *H. saltator*, the switch from non-reproductive worker to gamergate is accompanied by a reduced sensitivity to several CHCs, which could potentially reduce the mutually inhibitory or self-inhibitory effects of QPs on the gamergates (Ghaninia et al. 2017).

Axons of ORNs project to antennal lobe glomeruli in the brain. Caste- and sex-specificities in the chemosensory system have been investigated in *C. floridanus* (Zube and Rössler 2008), *Camponotus japonica* (Nakanishi et al. 2010), *Atta vollenweideri* (Kelber et al. 2010), and *Ooceraea (Cerapachys) biroi* (McKenzie et al. 2016). These studies have revealed substantial differences in the number, connectivity, and innervation of the female-specific T6 cluster of glomeruli which are innervated by ORNs of basiconic sensilla. These differences are likely to underlie QP-dependent differences in olfactory-guided behavior and learning. Each ORN usually expresses a single olfactory receptor (OR) along with an obligate odorant receptor co-receptor (ORCO). ORs and ORCO are members of the large family of 7-transmembrane receptors, and form functional heteromeric ligand-gated ion channels in the ORN membrane (Jones et al. 2007; Larsson et al. 2004; Sato et al. 2008). ORs provide

the coding specificity to the OR-ORCO complex and have broad ligand specificities, allowing the detection of a chemical (CHCs or other odorants) to be transmitted via an electric signal to the antennal lobes. Unlike other receptor types (i.e. gustatory receptors, ionotropic receptors, and pickpocket channels), the repertoire of ORs in ants is large (McKenzie et al. 2016; Oxley et al. 2014; Smith et al. 2011a, b; Zhou et al. 2012). In *H. saltator* (Pask et al. 2017) and *O. biroi* (McKenzie et al. 2016), most of the expressed OR genes that are prevalent in worker antenna belong to the 9-exon subfamily of OR genes. Indeed, several evolutionary studies have highlighted the great genetic expansion of the 9-exon subfamily of ORs that mirrors the diversity of CHCs production (Smith et al. 2011a, b; Zhou et al. 2012). By expressing 22 representatives of this OR subfamily from *H. saltator* in *D. melanogaster*, Pask et al. (2017) specifically identified HsOR263 as the putative detector of a fertility signal due to its strong response to both gamergate extract and the predicted QP component 13, 23-DiMeC37. There is less information available regarding ORCO, but studies of ORCO mutants in two ant species have revealed behavioral phenotypes consistent with the loss of pheromone sensing in various contexts. In addition, mutants had dramatically fewer ORNs and antennal lobe glomeruli (*H. saltator*, Tribble et al. 2017; *O. biroi*, Yan et al. 2017). Together, these studies suggest that ORNs and their associated molecular receptors (OR, ORCO) play a central role in detecting QPs.

### Hormonal Effects of QPs

There is a considerable lack of knowledge regarding the physiological and gene-regulatory events that follow signal transduction triggered by QP perception. However, several lines of evidence suggest that JH and biogenic amines play a central role in QP-mediated effects. First, the effects of QPs on worker ovarian development and larval caste can be mimicked by topical application of methoprene, a JH analogue (Edwards 1987; Penick and Liebig 2012; Vargo 1992; Vargo and Laurel 1994). In a social wasp, a recent study has shown that this treatment also affects CHC synthesis (Oliveira et al. 2017). Moreover, in the fire ant, experimental ablation of corpora allata, which release JH in the hemolymph, prevents dealation in the absence of a queen (Barker 1978). This effect was further shown to be mediated by an increase in dopamine, which stimulates JH secretions in other insect species (Boulay et al. 2001). Similarly, in *H. saltator* gamergates tend to have a greater level of brain dopamine than sterile workers (Penick et al. 2014). However, the role of biogenic amines is probably much more complex. In fire ant workers, QP was shown to provoke an increase in octopamine levels in the brain, which may be related to increased worker aggressiveness in queenright conditions (Vander Meer et al. 2008). Fire ant workers have no ovaries and no variation in dopamine has

been found with regard to the presence of a queen. However, in another species (*Streblognathus peetersi*), Cuvillier-Hot and Lenoir (2006) found that high-ranking workers with developed ovaries had less dopamine and more octopamine than low-ranking workers did.

### Molecular Effects of QPs

Very few studies have analyzed the specific molecular effects of QPs in adult individuals. Of the various mechanisms potentially promoted by QPs, epigenetic regulation of transcription, methylation, and RNA editing deserve special attention. In adult fire ants, Manfredini et al. (2014) observed differences in gene expression between foragers and non-foragers in *S. invicta* under queenright conditions, but these differences disappeared in queenless conditions. This suggests that in the absence of the queen, a modification of task allocation among workers (foraging vs. non-foraging) may be driven by QPs. In *L. niger* and *L. flavus*, workers treated with synthetic 3-MeC31 (the putative QP) displayed transcriptional rates of DNA methyltransferase (dnmt1 and dnmt3) that were 16–30% higher than those of untreated workers. According to the authors, “these data provide evidence that adult *L. niger* and *L. flavus* workers change their DNA methylation profile in response to QPs” (Holman et al. 2016b). Although promising, these findings examined only whole body DNA methyltransferase expression, and the consequences of QP perception on the methylome remain unknown.

More results, although indirect, come from examinations of the potential effect of QPs on larval caste fate. A pioneering expression study in *Cardiocondyla obscurior* compared gene expression between queenright larvae and larvae derived from eggs that were treated with methoprene which had previously been shown to induce development into gyne (Schrempf and Heinze 2006). The results showed that queen absence or presence during larval development affected several transcription factors and complex patterns of gene regulation associated with sphingolipid metabolism, which together may promote the larval developmental switch (Suefuji et al. 2008). These significant caste-dependent expression differences emerge early in larval development, and lay the foundation for the extensive morphological reorganization that occurs during pupal metamorphosis (Schrader et al. 2015). In a later study, Klein et al. (2016) examined the expression dataset of Schrader et al. (2015) using DEXseq to find exons that were differentially expressed among sexes or morphs, and found 179 exons of 91 genes with sex-biased expression. Of the 91 genes, 8 displayed both sex-specific and morph-specific alternative splicing, indicating that the differential splicing of these genes between worker and queen larvae. Among these was the transcription factor *doublesex* which promotes, through alternative splicing, differential cell JH sensitivity in developing tissues in stag beetles (Gotoh et al. 2014).

### Towards an Integrative Understanding of Ant QPs

Although the controversy between queen control and queen signal is sometimes still presented as an open question in ants, the debate is much less intense than twenty-five years ago. Most recent studies infer that queens signal their fertility or caste status to workers who respond in a way that increases their own inclusive fitness. However, a definitive test of the signaling hypothesis should also reject the alternative hypothesis of queen control (Keller 2009). For example, queens may manipulate workers by targeting a chemosensory system that workers use for another function (e.g. nestmate recognition). Such utilization of a pre-existing system in the target individuals may prevent them to escape control without losing the other important function. Yet, our knowledge of the chemical nature of QPs and of their functional effects on workers and brood are still insufficient to fill this gap. Indeed, many questions remain.

### How Are QPs Transmitted Within Complex Ant Nests?

In nature, ant colonies can be extremely populous and live in very complex networks of subterranean chambers or arboreal nests. In monogynous colonies, the queen needs to continuously produce enough pheromone to signal her presence to all colony members. However, the QP should also be sufficiently ephemeral for workers to rapidly be informed of her absence. Some authors have suggested that QPs are transmitted through social contacts among the workers (Passera, 1980) or through the intermediary of eggs, which act as messengers (Edwards 1991a; Endler et al. 2004; Seeley 1979). Further studies must be conducted in more species to test the role of queen eggs in the inhibition of adult egg-laying and gyne production.

### What Is the Role of QPs in Queen-Queen Competition?

So far, a single case of direct queen-queen competition has been reported, in *S. invicta* (Vargo 1992). This result is important but still awaits other experimental confirmation in red fire ants and in other polygynous species. Moreover, it is not clear why polygynous queens refrain from laying eggs in the presence of other queens.

### Is the QP a Single or a Blend of CHCs? What Is the Role of Glandular Secretions?

There is now sufficient information in many species to implicate CHCs in fertility signaling. However, so far, the ability of CHCs to inhibit worker ovarian development has only been reported in three species. Moreover, even though one compound may have a major role, QPs are likely to be complex mixtures of chemicals, each with a small effect. Further



studies should therefore attempt to identify QPs in a greater number of species. To this end, the publication of negative results should be encouraged.

### Is the QP Phylogenetically Conserved?

This question is totally unresolved. In ants, and most arthropods, cuticular chemistry is highly conserved: the cuticle is mostly, but not exclusively, composed of long-chain hydrocarbons whose primary function is protection against desiccation. These CHCs have been co-opted for multiple functions in communication, and in ants they serve in nestmate recognition, sexual interactions, and as QPs. The evolution of ant CHC profiles has undoubtedly been influenced by interactions among these different functions. Results so far tend to support the existence of great structural diversity in ant queen-specific CHCs. Whether this diversity is constrained by phylogeny and/or other CHC functions, however, is unknown.

### What Are the Molecular Targets of QPs?

We know very little of the molecular effects of QPs. Although the central role of JH and biogenic amines is globally well established, we still have very little information on the signaling routes that are triggered by QPs and how they generate multiple effects on ant physiology and behavior. A major question is whether QPs can be perceived by larvae and affect their development independently of changes in worker behavior. Moreover, although QPs have been hypothesized to affect larval caste fate through nutrition-mediated, JH-dependent processes, the functional link between QPs, worker behavior and physiology, nutrition, and JH signaling is mostly unknown.

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